

## REMARKS

Claims 17, 19, 24, 25, 30-32, 34, 39-43 and 45-49 were pending prior to this response. Page 2 of the Office Action mistakenly states that claims "20-32" are pending. Applicants assume that this error is typographical in nature and base the response on the assumption that the Examiner intended "30-32" instead. By the present communication, claims 3-9 and 12 have been cancelled without prejudice, and claims 17, 19, 25, 29-32, 39, 40, 42, 43, 45, 47 and 49 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and originally filed claims. Accordingly, claims 17, 19, 24-25, 29-32, 34, 39-43, 45-49 are currently pending.

### **The Objection to Claim 47**

Claim 47 is objected to as containing a typographical error in ending with two periods. To overcome the objection to claim 47, one of the periods at the end of the sentence has been deleted. Accordingly, Applicants respectfully request reconsideration and withdrawal of the objection to claim 47.

### **The Rejection Under 35 U.S.C. §112, Second Paragraph**

Applicants respectfully traverse the rejection of claims 3, 9-12, 71, 19, 24, 25, 29-32, 34 and 47-49 under 35 U.S.C. §112, second paragraph as allegedly being indefinite. With regard to claims 3 and 9-12, the Examiner asserts that the claims are dependent from base claims that are cancelled, making it unclear to what subject matter the claims are directed. To overcome the rejection, by the present communication claims 3 and 9-12 have been cancelled without prejudice, rendering the rejection moot.

With regard to independent claim 17, the Examiner asserts that the referent of the phrase "which cells" is unclear. By the present communication, claim 17 has been amended, as suggested by the Examiner by identifying "early attaching cells" as the referent of by modifying the phrase at issue to "which early attaching cells". In addition the Examiner asserts that claim 17

is indefinite as to whether the claim is directed to in vivo, ex vivo or in vitro transfection. For clarity, claim 17 has been amended to require that transfection of the early attaching cells is conducted "in vitro", and not by delivery of a vector into the bone marrow in vivo. In view of these amendments, Applicants submit that the metes and bounds of claim 17 has been clarified and withdrawal of the rejection is respectfully requested.

With regard to claim 25, the Examiner asserts that the metes and bounds of the claim is allegedly indeterminable because the phrase "culturing of the bone marrow" lacks sufficient antecedent support in base claim 17. To overcome the grounds for this rejection, claim 25 has been amended to recite: "...filtering the bone marrow and culturing the bone marrow to obtain the early attaching cells."

With regard to claims 29-32, the Examiner asserts that the phrase "the agent" lacks sufficient antecedent support in base claim 17. To overcome these grounds for rejection, claims 29-32 have been amended to replace "the agent" with the phrase "the angiogenic factor", which phrase is provided antecedent basis in claim 17 by the phrase "one or more angiogenic factors". In addition, the Examiner asserts that the meaning of the phrase "the composition" in claim 49 is unclear, allegedly due to the presence in claim 17 of multiple possible antecedents, such as "cells, bone marrow, adenoviral vectors" (Office Action, page 6). To overcome the grounds for the rejection, by the present communication dependency claim 49 has been changed to claim 47, and claim 47 has been amended to recite "injecting a composition comprising the one or more angiogenic factors in the conditioned medium along with the transfected early attaching cells". Thus, the phrase "a composition comprising" in claim 47 provides proper antecedent basis for "the composition" in claim 49.

In view of the above amendments, Applicants submit that, as currently amended, claims 17, 19, 24, 25, 29-32, 34 and 47-49 meet all requirements under 35 U.S.C. § 112, second paragraph, and reconsideration and withdrawal of the rejection thereof is respectfully requested.

**The Rejections Under 35 U.S.C. § 103**

A. Applicants respectfully traverse the rejection of claims 39-42 under 35 U.S.C. § 103 as being unpatentable over Iwaguro et al. (*Circulation*, 2002; 105:732-38), in view of Hamawy et al. (*Curr. Opin. Cardiol.* 1999; 14:515-22), and further in view of Hristov et al. (*Arterio. Thromb. Vasc. Biol.* 2003; 23:1185-89 and Tomita et al. (*Circulation*, 1999; 100 (Supp. II):247-56).

With regard to claims 38-42, the Examiner bases the combination of Igwaguro et al. and Hristov et al.) in the rejection on the assumption that EPCs whether obtained from bone marrow (Igwaguro et al.) or from peripheral circulation (Hristov et al.) share the “intrinsic” characteristic of being adherent in culture. However, neither the references themselves nor the comments of the Examiner provide, respectively, a suggestion or reasoning supporting the Examiner’s assumption that the two sources produce early attaching cells with equivalent characteristics and potentialities, other than the statement that EPCs obtained from peripheral blood are ultimately obtained from bone marrow, as evidenced by Iwaguro et al and Hristov et al. In fact, the Examiner acknowledges that EPCs are only one component of the early attaching cells that are obtained from culture of bone marrow cells.

Applicants submit that those of skill in the art at the time the application was filed would not have been motivated by the teaching of Hristov et al. regarding EPCs from peripheral blood to substitute therefore early attaching cells from bone marrow. For example, the EPCs from peripheral blood are exceedingly rare and are separated from peripheral blood by culturing whereas, in Applicants’ invention all the cells from bone marrow are used as the starting point and, as acknowledged by the Examiner, the early attaching cells from bone marrow culture include, but are not limited to EPCs.

The disclosure of Hristov et al. is absolutely silent regarding any advantages for angiogenesis in ischemic tissue that could be obtained by cell types other than EPCs present in bone marrow cells or in the early attaching cells obtained from bone marrow. Particularly Hristov et al. are silent regarding the natural angiogenic factors that would be expressed by such additional types of bone marrow cells in culture or when injected into ischemic tissue.

Therefore, Applicants submit that the combined teachings of the references cited, including the teaching of Iwagura regarding ex vivo expansion and transfection of EPCs with an angiogenic factor would not suggest the invention compositions, as recited by currently amended claims 39-42.

In particular, Applicants submit that the Examiner has provided no reasoning for the assumption that the teaching of Hristov et al. regarding EPCs obtained from peripheral blood would have suggested to those of skill in the art to formulate a composition, as required by currently amended claim 42, "consisting essentially of" CD34<sup>+</sup>/CD35<sup>-</sup> cells obtained from bone marrow early attaching cells and transfected with a vector encoding an angiogenic factor as having similar properties and uses, e.g., treatment of ischemic tissue in heart or limb to restore blood flow.

Particularly with regard to claim 42, the Examiner asserts that the limitation for the step of stimulation of transfected bone marrow cells with hypoxia is deemed of little moment in determining patentability of the claimed composition absent evidence that the product so "manipulated" is necessarily distinguished by the manipulation (Office Action page 7). However, Applicants submit that the step of in vitro stimulation of the cells, as required in claim 42, definitely changes the product produced and creates a product distinct from the one identified by the Examiner (i.e., "early attaching cells obtained from bone marrow, transfected with adenoviral vector") because the step of stimulating the transfected cells causes a change in the product produced.

As Applicants teach in the Specification, stimulation of the cells in bone marrow by hypoxia results in a different product than a composition containing bone marrow cells that are not stimulated by hypoxia, whether or not the bone marrow cells have been transfected with a transgene. For example, at paragraph [0024] of the Specification, Applicants teach the following:

[0024] One angiogenesis-promoting factor that most likely participates in initiating angiogenesis in response to ischemia is HIF-1, a potent transcription factor that binds to and stimulates the promoter of several genes involved in responses to hypoxia. Induction and activation of HIF-1 is tightly controlled by tissue pO<sub>2</sub>. HIF-1 expression increases exponentially as pO<sub>2</sub> decreases, thereby providing a positive feedback loop by which a decrease in pO<sub>2</sub> causes an increase in expression of gene products that serve as an adaptive

response to a low oxygen environment. Activation of HIF-1 leads, for example, to the induction of erythropoietin, genes involved in glycolysis, and to the expression of VEGF. HIF-1 is thought to also modulate the expression of many other genes that participate in the adaptive response to low  $pO_2$  levels. HIF-1 regulates levels of proteins involved in the response to hypoxia by transcriptional regulation of genes responding to low  $pO_2$ , which genes have short DNA sequences within the promoter or enhancer regions that contain HIF-1 binding sites, designated as hypoxia responsive elements (HRE).

Applicants for the first time teach that the HIF-1 gene naturally present in bone marrow cells can be stimulated *ex vivo* by hypoxia to increase expression of natural gene products, such as erythropoietin, VEGF, and genes involved in glycolysis, to levels not found in a similar bone marrow cell composition in which the cells have not been stimulated or activated *ex vivo* by hypoxia (e.g. *in vitro*), whether or not the bone marrow cells have been transfected with a transgene. Accordingly, it is the position of the Applicants that the product itself described in claim 42 is a different product than that required in the other composition claims because of the inherent presence in the composition of the products of natural genes found in early attaching cells obtained from bone marrow, which natural products are either substantially lacking in a composition comprising such cells that have not been so stimulated (e.g., HIF-1), or are present at lower levels (e.g. VEGF), than in the invention hypoxia stimulated bone marrow cell composition.

Accordingly, Applicants submit that the product required by claim 42 is patentable over the art cited in the Office Action. The Examiner has not pointed out in any of the references, or in any combination of the references cited, a discussion of the effect of *in vitro* or *ex vivo* hypoxia stimulation of bone marrow cells, such as early attaching bone marrow cells. Consequently, Applicants submit that those of skill in the art would not be motivated by the cited art or any combination thereof to obtain a bone marrow cell composition as claimed in claim 42, which requires a step in fabrication of the composition involving *in vitro* stimulation of the transfected bone marrow cells by subjection to hypoxic conditions, such as those disclosed by Applicants.

Even if those of skill in the art would have been motivated by knowledge of the art concerning the effect of hypoxia on certain cell types *in vivo*, to try out the effect of hypoxia on bone marrow cells *in vitro*, there is no suggestion as to how to succeed or whether the effect

would be beneficial. Moreover, "obvious to try" has never been the standard for determining obviousness under 35 U.S.C. § 103.

Accordingly, Applicants submit that prima facie obviousness of the composition of claims 39-42 is not established over the art cited under 35 U.S.C. § 103 and reconsideration and withdrawal of the rejection is respectfully requested.

B. Applicants respectfully traverse the rejection of claims 17, 24, 29, 30, 34, 39-43 and 46-49 under 35 U.S.C. § 103 as being unpatentable over Iwaguro et al., in view of Hamawy et al. as above, or Isner et al. (US Patent No. 6,569,428, and further in view of Hristov et al. (*Arterio. Thromb. Vasc. Biol.* 2003; 23:1185-89 and Tomita et al. (*Circulation*, 1999; 100 (Supp. II):247-56). Applicants submit that the invention methods, as defined by currently amended claim 17, distinguish over the cited art by reciting:

A method for enhancing collateral blood vessel formation in heart or limb muscle tissue, said method comprising:

directly injecting into a site of impaired blood flow in heart or limb muscle tissue an effective amount of early attaching cells obtained from autologous bone marrow, which early attaching cells have been transfected *in vitro* with an adenoviral vector comprising a polynucleotide encoding one or more angiogenic factors selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS).

The arguments above regarding the Iwaguro-Hamawy-Hristov combination of references as applied to the invention composition apply as well here and are incorporated in this discussion of the patentability of the invention methods using the invention composition.

In addition, Applicants disagree with the Examiner's assumption that the term "directly injected" as used in the claims to indicate needle injection into target ischemic muscle sites is suggested by Iwaguro's description of a bolus systemic injection of transfected EPCs through the tail vein (Office Action, page 11). The cited art contains no suggestion that would motivate those

of skill in the art to modify the disclosure of the references to substitute for systemic bolus injection of EPCs the direct injection into ischemic muscle tissue in heart or limb of transfected early attaching cells obtained from bone marrow. The Examiner has cited no reference that contemplates direct injection of EPCs into ischemic heart or limb tissue for the specific purpose of enhancing development of collateral vessels therein. Rather, the Examiner relies upon Isner et al. as suggesting bone marrow as the source of EPCs, but Isner discloses that EPCs are separated using anti-CD34 antibodies and Isner's description is focused on obtaining EPCs from peripheral blood, not bone marrow. The Examiner also relies upon Isner et al. as disclosing site-directed administration by catheter, but Isner et al. fails to mention or suggest direct injection into ischemic tissue in heart or limb for the purpose of enhancing collateral vessel development in ischemic tissue because Isner et al. states that the prior art method can be used to treat "unregulated angiogenesis or blood vessel injury" (Isner et al. Col. 7, line 18). The first of Isner's disclosed uses, treating "unregulated angiogenesis", teaches away from the invention since development of collateral blood vessels in ischemic heart or limb muscle is the opposite of curbing unregulated angiogenesis. The second use disclosed by Isner, "blood vessel injury," is too vague to suggest specifically the claimed result of the invention methods, enhanced development of collateral vessels in ischemic muscle in heart or limb. If anything, treatment of blood vessel injury, as described by Isner suggests repair of damaged blood vessels.

In fact, Applicants submit that none of the cited art, when taken individually or when viewed in the light of one another suggests anything about development of collateral vessels in ischemia-damaged heart or limb muscle. Angiogenesis is a term implying formation of new blood vessels. On the other hand, as Applicants have argued throughout the prosecution of this invention, the present invention is drawn to development of immature blood vessels already present in the muscle tissue, not to random generation of new vessels. Thus, the particular angiogenic factors required in the claims are specifically selected to promote development of immature blood vessels in ischemia-damaged muscle tissue, not to promote unregulated angiogenesis, which can itself be problematic, or to repair damaged vessels.

In particular, with regard to claim 49, Applicants disagree with the Examiner's assertion that the recitation of direct injection of effective amounts of "about 0.2 to about 0.5 ml" of the composition in from about 12 to 25 sites" can be interpreted to mean in "from 1 to 40 sites" (Office Action, page 11). Applicants submit that it is disingenuous to interpret the meaning of claim 49 as equivalent to saying that the injection is made at a single injection site by interpreting the term "about" as being "broad and open-ended." It well established in patent law that the meaning of the term "about" in claim language is not "broad and open-ended" but is definite. Moreover, the metes and bounds of a claimed range in which the term "about" appears is determined in the context of the invention itself. The Examiner has provided absolutely no reason in support of the arbitrary statement that "about 12 to 25 sites" means "from 1 to 40 sites".

Therefore, Applicants submit that the descriptions of Iwaguro et al., and Hamawy et al. regarding gene therapy using various angiogenic factors and the description of Tomita et al. regarding use of an anticoagulant in a composition of bone marrow-derived cells do not cure the above-described deficiencies in the cited art for suggesting the claims drawn to the invention methods and are insufficient to motivate those of skill in the art to modify the cited art to arrive at the claimed invention methods for enhancing development of collateral vessel development in ischemic heart or limb tissue.

Accordingly, Applicants submit that prima facie obviousness of claims 17, 24, 29, 30, 34, 39-43 and 46-49 is not established over the Iwaguro, Hamawy-Isner-Hristov-Tomita combination of references and reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 are respectfully requested.

C. Applicants respectfully traverse the rejection of claims 17, 24, 29, 30, 34, 39-43 and 46-49 under 35 U.S.C. § 103 as being unpatentable over Iwaguro et al. in view of Hamawy et al. or Isner et al., and further in view of Hristov et al. as above and further in view of Furcht et al. (U.S. 2005/0181502 A1). The remarks above concerning the Iwaguro, Hamawy-Isner-Hristov-Tomita apply equally and are incorporated here. The Examiner acknowledges that none of these references explicitly teach that early attaching cells are CD34/CD45<sup>+</sup> cells, but asserts: "the EPCs



as taught by Iwaguro and Hristov would reasonably be expected to contain some cells that are CD34/CD45" (Office Action, page 14).

However, currently amended claim 45 requires that the bone marrow derived cells "consist essentially of marrow stromal cells", which marrow stromal cells the Examiner acknowledges are defined in the Specification as "CD34 minus/CD45 (CD34/CD45)minus cells (Office Action, page 13). The Examiner relies upon Furcht as disclosing such cells as beneficial in therapeutic applications, because as progenitor cells, they are able to differentiate into a wide variety of cell types, including endothelial cells. However, Applicants submit that Furcht is silent regarding use of such cells, when transfected with an adenoviral vector encoding an angiogenic factor, for direct injection into ischemic muscle of heart or limb to enhance development of collateral vessels therein. Applicants respectfully submit that there is nothing in the disclosure of the Iwaguro-Hamawy-Isner-Hristov-Furcht combination of references, either individually or taken together, that suggests transfecting such a sub-population of the early attaching cells obtained from bone marrow for use in a method for enhancing development of collateral blood vessel formation in ischemic heart or limb muscle tissue.

Therefore, Applicants respectfully submit that prima facie obviousness is not established over the Iwaguro-Hamawy-Isner-Hristov-Furcht combination of references and reconsideration and withdrawal of the rejection is respectfully requested.

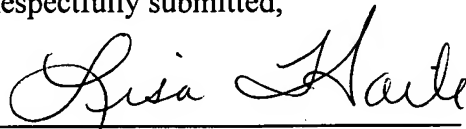
D. Applicants respectfully traverse the rejection of claims 17, 24, 29, 30, 34, 39-43 and 46-49 under 35 U.S.C. § 103 as being unpatentable over Iwaguro et al. in view of Hamawy et al. or Isner et al., and further in view of Hristov et al., Furcht et al., further in view of Smith et al. (Arter. Thromb Vasc. Biol. 2002 2:1279-85) and Li et al. (Nat. Med. 2000; 6:49-55) The remarks above concerning the Iwaguro, Hamawy-Isner-Hristov-Tomita-Furcht combination of references apply equally and are incorporated here. Additionally the Examiner relies upon Smith et al. and Li et al. as disclosing that NOS or PR39 can be used in an adenoviral vector to treat muscle ischemia as taught by Iwaguro and/or Isner. Applicants submit however that in view of the deficiencies of the cited art for suggesting the invention methods to those of skill in the art are not overcome by the

disclosures of Smith and/or Li because the teachings of both references can suggest no more than modification of the content of an adenoviral vector to encode specific angiogenesis factors (i.e., NOS or PR39) that when viewed in the context of Iwaguro and/or Isner might be useful to "promote angiogenesis", as asserted by the Examiner. However, the combination of art cited fails to suggest transfecting the population of early attaching cells obtained from bone marrow with any one of the particular claimed angiogenesis factor for direct injection into ischemic heart or limb muscle to enhance development of collateral vessels therein because the cited art is silent regarding enhancement of collateral blood vessel formation. Further, Applicants submit that due to this major deficiency in the cited art, those of skill in the art would not be motivated by the references, and if motivated would not understand how to arrive at the claimed methods for enhancing development of collateral blood flow in ischemic heart or limb muscle.

In view of the above amendments and remarks, Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call Applicants' representative Lisa A. Haile so that a prompt disposition of this application can be achieved.

A check in the amount of \$225.00 is enclosed to cover the Two Month Extension of Time fee due. No additional fee is believed due in connection with this submission. In the event that fees are due, please charge our Deposit Account No. 07-1896, referencing the above-identified docket number.

Respectfully submitted,



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